Enzyme Loading Experiments with Washed Corn Fiber

Tammy Kay Hayward
June 1995

Experiment **Run Dates:** February 22-April 20, 1995
Researcher: Tammy Kay Hayward
Oral Presentation: March and April 1995 CRADA meetings
Lab Book References: #1092, 114-117 and #1651,008-010

Objectives: To generate data on glucose release from cellulose present in washed Corn Fiber solids. To determine the effect of cellulase enzyme loading on the saccharification and SSF of the above. Data to be used in modeling the process.

Materials and Methods:
Pretreated corn fiber was diluted in D.I. water, adjusted to pH 5 with lime and then filtered onto a paper filter under vacuum. The wet solid cake was then washed extensively with water until the filter liquid was colorless. (liquor starts as a dark brown). The goal of this was to remove the glucose and other free sugars from the substrate. Cellulase enzymes are very sensitive to feed-back inhibition and this modeling study would be served best with an extensively washed cellulosic substrate with no free glucose at time zero. The wet washed solids cake was placed in a plastic bag and kneaded into a homogenous yellow corn clay and then stored in the refrigerator. These washed solids would become very moldy over a period of 2 weeks, so solids had to be freshly washed. The saccharifications were performed at 5% solids. the SSFs were performed at both 5 and 10%solids.

Cellulase Enzyme: The cellulase enzyme used in the experiments was CPN PDU lot with a measured Filter Paper Activity of 70 units per mL. The beta-glucosidase activity is 231 units per milliliter per minute. Both activities were measured on the filter sterile enzyme by Bill Adney. The enzyme was diluted ten-fold in *sterile* D.I. water prior to use. No precipitate formed with this preparation. The undiluted cellulase is suspended in 280 g/L sucrose.

Media:-Corn Steep Liquor from Grain Products Corporation was diluted ten-fold in D.I. water, adjusted to pH 5 with ammonium hydroxide, autoclaved for 30 minutes and then filter sterilized. The washed ECF solids were suspended in 0.5% CSL for pH control in saccharification. This low level improves the resolution of glucose and cellobiose peaks on the HPLC. The SSFs were conducted in 1% Yeast Extract, 2% Peptone at pH 5 in order to insure enough nutrients for optimal fermentation.

Conditions: All experiments were performed in shakers set at 34°C, 150rpm using washed extruded corn fiber solids.

Saccharifications: Points were taken at time zero, and then frequently in the first day and tampering off for six days. Each whole slurry sample was boiled to denature the enzyme and centrifuged to remove the solids, diluted if necessary and read on the YSI. Liquid samples were submitted to the CAT task for glucose and cellobiose by HPLC.

SSFs: In contrast to the above, samples were not taken at time zero. No samples are taken during the first day of SSF. The samples are not boiled to avoid loss of ethanol. They are centrifuged to remove the solid substrate and read on the YSI for both glucose and ethanol.

Experimental Designs:
All performed in duplicate shake flasks
Experiment 1: Saccharifications enzyme loadings 2.3-18.5 FPU
Experiment 2: Saccharifications enzyme loadings 10-40 FPU
Experiment 3: SSFs enzyme loadings 5-20 FPU

Results:

Saccharification I Glucose and cellobiose were produced at all enzyme loadings. Figure 1 is a graph of glucose release over time.

Saccharification II

Figure 2 shows the glucose release for the higher enzyme loadings in the range of 10 FPU to 40 FPU per gram of cellulose in the washed corn fiber solids. Theoretical glucose yield approaches 90% theoretical (based on cellulose in washed solids) in both experiments around 20 FPUs. At the low enzyme loading the levels of glucose and cellobiose are almost equal. In contrast, at the high enzyme loading the level of cellobiose is considerably lower than glucose, with the highest cellobiose level at the early. 4 hour time point (spike). See figure 3 for a glucose and cellobiose profile. Both glucose and cellobiose concentration were added together and called total sugars. The relationship between enzyme loading (2.3 and 40 FPU/g) and total sugars and time trends is depicted in figure 4. The non-linear relationship between enzyme loading and sugar release in shown in figure 5. The glucose and cellobiose data is attached as CAT task reports in the appendix. Tammy

SSF

Again, the theoretical yield of ethanol is near 100% based on cellulose in the washed solids. The flasks at the lowest enzyme loading (5 FPU) and the highest solids concentration (10%) show a sever decrease in ethanol yield. Sampling was also difficult with the low enzyme flask. At 10 FPU, there was very little effect of solids level as both the 10% solids and the 5% solids produced similar yields of ethanol. See figure 6 for YSI ethanol and SSF time relationships. The last time points were also run by the CAT task for ethanol concentration. The CAT task report is in the appendix. The data is also in the Excel spreadsheet.

Conclusions:

A significant mount of data was generated by three separate experiments for modeling the effects of enzyme loading on washed. corn fiber. The washed solids allowed us to explore the effect of the PDU enzyme on the cellulosic portion of the substrate without the background of monomeric and oligomeric sugars naturally in whole slurry corn fiber. This reduced the amount of feedback inhibition on the enzymes and improved the analytical resolution of the sugars released during saccharification. In both the saccharification and the SSF experiments, the amount of glucose or ethanol produced was above theoretical suggesting that the cellulose content number is too low. or the glucose and ethanol numbers are too high. To try to solve this problem, a another experiment was designed to try to close the mass balance on this washed, extruded corn fiber. This experiment was named the Detailed Saccharification and will have its own report.